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Percentage and Concentration of the Antibiotic Polymyxin in Extracts of Four Species Belonging to the Genus *Bacillus*

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Abstract

The study included the determination of the antibiotic (Polymyxin) and its proportions in the organic extracts of marine species of the genus *Bacillus*, namely (*B. cereus, B. subtilis, B. circulans, B. polymyxa*), which were isolated from beach sediment samples from the Afamia region with geographical coordinates (35°76'19.1"N; 35°54'19.3"E) It is an open area affected by tourist activities, sewage canals, and ship waste. To obtain organic extracts of bacterial isolates, the optimal conditions for separating and purifying these antibiotics were determined at the lowest economic cost, given the importance of these antibiotics in the pharmaceutical industries and their effective impact on pathogenic microbes and cancer cells. The results showed that after separating the low-density liquid layer (the upper layer) from the high-density liquid layer (the lower layer) and measuring the absorbance using a spectrophotometer for each antibiotic, the optimal conditions for purifying and isolating antibiotics were at a centrifugation speed of 4000 rpm, a centrifugation time of (30) minutes, and a temperature of -5°C. The results showed that the percentage of the antibiotic polymyxin upon centrifugation at -5°C for 30 minutes was 91.30% for the extract of *B. subtilis*, 97.91% for the extract of *B. polymyxa* and 88.23% for the extract of *B. circulans*.

Keywords: *Bacillus*; Antibiotic; Spectrophotometer

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Introduction

Marine microorganisms have attracted the attention of many researchers due to their potential use in biotechnology applications. The rapid development in marine chemistry over the past fifteen years has led to the discovery of a large number of new chemical compounds with previously unknown pharmacological properties [10]. However, research on these compounds only began effectively in the middle of the last century and encompassed all forms of life in the marine environment [2]. By 2014, researchers had isolated approximately 25,000 chemical compounds from marine organisms (microorganisms, phytoplankton, and green algae). Red spores, sponges, corals, mollusks, echinoderms, etc.), and 20,000 of these compounds possess bioactive biochemical properties that have become a source of pharmaceutical drugs [3]. These natural products include molecules with various biological activities such as antimicrobials, antioxidants, vasodilators, and antidiabetic agents [9]. Marine microorganisms are of great importance as potential sources for developing drugs such as new chemotherapeutic agents, due to the novel antimicrobials they produce to combat diseases caused by multidrug-resistant pathogens that have become a major threat to public health. Approximately 40-50% of commercial drugs are produced starting from natural chemical products [4, 17].

Antibiotics produced by bacteria of the genus *Bacillus*

Bacillus species are characterized by their resistance to adverse environmental conditions through spore production [14]. Furthermore, these bacteria are distinguished from others by their production of large quantities of primary and secondary metabolites (such as antibiotics, enzymes, and organic acids). The most important characteristic of antibiotics produced by this genus is that they are peptide antibiotics, which may be linear, but most are circular [7, 16]. *Bacillus* bacteria produce numerous peptide antibiotics, which are effective against a wide range of pathogenic bacteria [15].

Characteristics of Polypeptide Antibiotics

One of the most important types of antibiotics produced by bacteria of the genus *Bacillus* belongs to the polypeptide class [12]. Polymyxin is a key example of a polypeptide antibiotic produced by *Bacillus* species and is characterized by the following the antibiotic polymyxin:

Chemical formula: C56H100N16O17S. Molecular mass: 1301.57 g/mol. Chemical structure: It is a polypeptide composed of 10 amino acids, six of which are alpha-gamma-diamine butyric acid (L-DAB). Spectrum of action: Polymyxin selectively affects Gram-positive and Gram-negative bacteria, as well as fungi [13]. Uses: Polymyxin can be used successfully in the treatment of various forms of eczema and other skin diseases. It is also used in the treatment of meningitis, urinary tract infections, and otitis externa. Mechanism of action: Polymyxin alters the permeability of the bacterial cell membrane by binding to a negatively charged site in the lipopolysaccharide layer [8].

The importance and objectives of the research

Given the importance of extracts of the genus *Bacillus* in the field of antibiotic and chemical compound production, and the scarcity of studies on types of the genus *Bacillus*, the aim of the study was to study the chemical composition of the organic extract of the genus *Bacillus* and to identify the biological importance and biological activity of the chemical compounds of the organic extract of the genus *Bacillus* that were isolated from the coastal marine sediments of the Apamea area in the city of Latakia.

Research Materials and Methods

The study was conducted on marine sediments from the Latakia city beach, specifically from the Apamea area (35°76'19.1"N; 35°54'19.3"E). This area represents an open area affected by tourism activities and sewage canals. Samples of the marine sediments were collected from the aforementioned site using sterile 500 ml glass containers and transported directly to the laboratories of the Higher Institute for Marine Research at Latakia University for microbiological analysis.

Laboratory study

Preparation of the organic extract of bacterial isolates: Marine coastal sediment samples were collected from the Afamia area using sterile 500 ml glass bottles, and were directly transferred to the laboratories of the Higher Institute for Marine Research, University of Latakia, to conduct the microbiological laboratory study. After obtaining marine isolates of different types of the genus *Bacillus*, which were typed, namely (*B. cereus, B. subtilis, B. circulans, B. polymyxa*) [11, 14].

The antibiotic polymyxin and its concentration in the methanolic extracts of four *Bacillus* species isolated from the Apamea region were determined because they exhibited higher efficacy than dichloromethane extracts from species isolated from other locations. Optimal conditions were established for the separation and purification of this antibiotic at the lowest economic cost [1, 5], given its importance in the pharmaceutical industry and its potent effect against pathogenic microbes and cancer cells. This is because the manufacture of some antibiotics requires secondary compounds involving various chemical groups; hence the importance of obtaining these compounds from their natural sources [6, 9].

The concentration of antibiotics was studied using a spectrophotometer according to the method of Hammod et al. [6]. The method involves the following steps: Extracts of *Bacillus* species were placed in a centrifuge (Eppendorf 5810r, Germany) at a centrifuge speed of 4000 rpm at a temperature of -5°C for different time periods of 10, 20, and 30 minutes. The lower-density liquid layer was separated from the lower-density layer. To determine the absorption wavelength of each antibiotic, the spectrophotometer was zeroed using the organic solvent methanol (as a control). The optimal wavelength was experimentally determined by scanning the wavelengths within the nm range (190-800 nm) of the antibiotic polymyxin to identify the wavelength at which the antibiotic exhibits its highest absorption, using a spectrophotometer. A standard series of antibiotics at different concentrations (0.01, 0.02, 0.05, 0.1 mg/ml) was used. To establish a standard curve and obtain the equation for calculating antibiotic concentrations and their ratios based on the absorbance values of these different concentrations, the concentration of each antibiotic in the organic extracts was determined in each of the two layers formed—the low-density liquid layer and the high-density lower layer—using a spectrophotometer. The percentage of each antibiotic in each isolate (purification percentage) was calculated using the following formula: (Antibiotic concentration in the separated layer ÷ Antibiotic concentration in the original sample) × 100 [5].

Results and Discussion

Isolation and Concentration of the Antibiotic Polymyxin from *Bacillus* Extracts: The wavelength of the antibiotic Polymyxin was determined using a spectrophotometer, yielding a value of 647 nm. The concentration of the antibiotic in *Bacillus* extracts isolated from the Afamia region during the summer was then determined, as it exhibited higher inhibitory activity against pathogenic bacteria and a broad spectrum of biological activity against various pathogenic bacteria and bioactive compounds, as demonstrated in previous studies.

The results showed that the total concentration of the antibiotic polymyxin in the organic extract of each of the four *Bacillus* species (*B. subtilis, B. circulans, and B. polymyxa*) was 2.3 mg/l for *B. subtilis*, 4.8 mg/l for *B. polymyxa,* and 1.7 mg/l for *B. circulans*. No concentration was recorded for *B. cereus* (Table 1).

When each phase (low-density liquid and high-density liquid) of the methanolic extract was studied during centrifugation at -5°C for different centrifugation times (10, 20, and 30 minutes), the results

Table 1: Concentrations of the antibiotic polymyxin for each of the liquid layers of low- and high-density extracts of the four species belonging to the genus *Bacillus* at temperatures of (5-) °C and different centrifugation periods (10, 20, 30) minutes.

The Concentration of the antibiotic polymyxin Estimated in mg/l				
	B. circulans	***B. polymyxa***	***B. subtilis***	***B. cereus***
Total concentration of the antibody in the abstract	1.7	4.8	2.3	
Centrifugation at (-5)°C for 10 minutes				
Low density liquid layer	0.1	2.6	1.5	
High density liquid layer	1.1	3.5	1.7	
Purification percentage	64.70%	66.03%	73.91%	
Sedimentation at temperature (-5)°C For a20 minute				
Low density liquid layer	0.4	2.8	1.6	
High density liquid layer	1.3	4.1	1.9	
Purification percentage	78.47%	77.35%	82.60%	
Sedimentation at temperature (-5)°C For a30 minute				
Low density liquid layer	0.1	2.3	1.8	
High density liquid layer	1.5	4.7	2.1	
Purification percentage	88.23%	97.91%	91.30%	

showed that:

Upon centrifugation at -5°C for 10 minutes, the low-density liquid layer showed a polymyxin concentration of 1.5 mg/l for *B. subtilis* extract, 2.6 mg/l for *B. polymyxa* extract, and 0.1 mg/l for *B. circulans* extract. For the high-density liquid layer, the polymyxin concentration was 1.7 mg/l for *B. subtilis* extract, 3.5 mg/l for *B. polymyxa* extract, and 1.1 mg/l for *B. circulans* extract (Table 1). The results showed that the percentage of the antibiotic polymyxin upon centrifugation at -5°C for 10 minutes was 73.91% for the extract of *B. subtilis*, 66.03% for the extract of *B. polymyxa*, and 64.70% for the extract of *B. circulans* (Table 1).

To determine the effect of centrifugation on antibiotic concentration in organic extracts, centrifugation was performed at 20 and 30 minutes at -5°C, and the results were as follows: Upon centrifugation at -5°C for 20 minutes, the low-density liquid layer showed that the polymyxin concentration was 1.6 mg/l for *B. subtilis* extract, 2.8 mg/l for *B. polymyxa* extract, and 0.4 mg/l for *B. circulans* extract (Table 1).

For the high-density liquid layer, the results showed that the polymyxin concentration in the bacterial extracts of *Bacillus* species was 1.9 mg/l for *B. subtilis* extract, 4.1 mg/l for *B. polymyxa* extract, and 1.3 mg/l for *B. circulans* extract (Table 1). The results showed that the percentage of polymyxin upon centrifugation at -5 °C for 20 minutes was 82.60% for the extract of *B. subtilis*, 77.35% for the extract of *B. polymyxa* and 78.47% for the extract of B. circulans (Table 1).

Upon centrifugation at -5°C for 30 minutes, the low-density liquid layer showed a polymyxin concentration of 1.8 mg/l for *B. subtilis* extract, 2.3 mg/l for *B. polymyxa* extract, and 0.1 mg/l for *B. circulans* extract. For the high-density liquid layer, the polymyxin concentration was 2.1 mg/l for *B. subtilis* extract, 4.7 mg/l for *B. polymyxa* extract, and 1.5 mg/l for *B. circulans* extract (Table 1). The results showed that the percentage of the antibiotic polymyxin upon centrifugation at -5 °C for 30 minutes was 91.30% for the extract of *B. subtilis*, 97.91% for the extract of *B. polymyxa* and 88.23% for the extract of *B. circulans.*

The results, after separating the low-density liquid layer (upper layer) from the high-density liquid layer (lower layer) and measuring the absorbance using a spectrophotometer for each antibiotic, showed that the optimal conditions for purifying and isolating the antibiotics were at a centrifugation speed of 4000 rpm, a centrifugation time of (30) minutes, and a temperature of -5°C. The concentration of the antibiotic in each layer was determined, and we observed that the separation rate of some antibiotics exceeded 95%. The highest percentage was recorded for the antibiotic polymyxin, reaching 97.91% for the extract of *B. polymyxa*.

Conclusions and Recommendations

The most important polypeptide antibiotics and their proportions produced by *Bacillus* species were identified as polymyxin. The highest concentration of polymyxin was recorded for the extract of *B. polymyxa* bacteria. The optimal temperature for isolating antibiotics from methanolic extracts of bacterial isolates was -5°C and a centrifugation time of 30 minutes. This type of research is currently at the forefront of marine biotechnology research, aiming to leverage its applications in extracting therapeutically and pharmaceutically valuable substances from marine organisms. It also seeks natural sources for producing broad-spectrum, non-traditional, natural antibiotics against common human pathogenic bacteria, with significantly fewer side effects than conventional and synthetic antibiotics. Furthermore, it is essential to utilize marine *Bacillus* bacteria, which possess significant medical and pharmaceutical value, at a low cost.

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